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Effects of Changes in Sodium Balance on Prostaglandin Synthesis and Prostaglandin E_2 9-Ketoreductase Activity in the Rat Kidney

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Summary: The in vitro synthesis of prostaglandins E_2 and $F_{2\alpha}$ by renal cortex, medulla and papilla was measured in normal rats and in rats receiving either a low or a high sodium intake for 14 days.

The production of both prostaglandins was unchanged in the cortex. In the medulla, both low and high sodium intakes led to a similar decrease in prostaglandin E_2 synthesis in vitro, but prostaglandin $F_{2\alpha}$ synthesis was unchanged. In the papilla, a low sodium intake increased prostaglandin E_2 synthesis.

The activity of prostaglandin E_2 9-ketoreductase, a cytosolic enzyme catalysing the conversion of prostaglandin E_2 to prostaglandin $F_{2\alpha}$, was unchanged in cortical preparations. In medullary slices, prostaglandin E_2 9-ketoreductase activity was decreased by both sodium depletion and loading. In the papilla, prostaglandin E_2 9-ketoreductase activity was slightly decreased by sodium loading and increased with sodium depletion.

These results obtained in the rat are at variance with findings in the rabbit. The role played by prostaglandin E_2 9-ketoreductase in the regulation of prostaglandin biosynthesis during changes of sodium balance remains controversial.

Introduction

A relationship between prostaglandins and tubular sodium handling by the kidney is suggested by several lines of evidence, such as the natriuretic effect of prostaglandins in vivo (1), the influence of prostaglandins on sodium transport in vitro (2), the frequent induction of sodium retention during inhibition of prostaglandin synthesis (3) and changes in the urinary excretion of different prostanoids which is associated with changes in sodium balance (4). However, the precise role of prostaglandins in this aspect of renal physiology remains controversial (5).

It has been suggested that the relative levels of prostaglandin E_2 and prostaglandin $F_{2\alpha}$ might be of importance in determining their physiological effect (4, 6). The prostaglandin E_2/prostaglandin $F_{2\alpha}$ ratio is considered to be regulated by both the synthesis and the catabolism of the two substances, as well as by the activity of the cytosolic enzyme, prostaglandin E_2 9-ketoreductase[1]), which catalyses the interconversion of prostaglandin E_2 and prostaglandin $F_{2\alpha}$ (7). Previous studies have demonstrated that prostaglandin

[1]) Enzymes
15-Hydroxyprostaglandin dehydrogenase (NAD^+); (5*Z*,13E)-(15*S*)-11α,15-Dihydroxy-9-oxoprost-13-enoate: NAD^+ 15-oxidoreductase (EC 1.1.1.141)
15-Hydroxyprostaglandin dehydrogenase ($NADP^+$); (13*E*)-(15*S*)-11α,15-Dihydroxy-9-oxoprost-13 enoate: $NADP^+$ 15-oxidoreductase (EC 1.1.1.197)
Prostaglandin H_2 E-isomerase; (5*Z*,13*E*)-(15*S*)-9α,11α-Epidioxy-15-hydroxyprosta-5,13-dienoate E-isomerase (EC 5.3.99.3)
Prostaglandin E_2 9-ketoreductase; (5*Z*,13*E*)-(15*S*)-9α,11α,15-Trihydroxy-prosta-5,13-dienoate: $NADP^+$ 11-oxidoreductase (EC 1.1.1.189)
Renin; Angiotensin forming enzyme (EC 3.4.23.15)

E_2 9-ketoreductase activity is influenced by the status of sodium balance in the rabbit kidney (6) and in isolated rat glomeruli (8). The present study was therefore designed to measure the production of prostaglandin E_2 and prostaglandin $F_{2\alpha}$ and the activity of prostaglandin E_2 9-ketoreductase by different compartments of the rat kidney in vitro after manipulation of sodium balance in vivo.

Materials and Methods

Experiments were performed on 15 female Long-Evans rats, weighing 227–333 g (mean 287 g). Five rats received a diet containing 3.5 g of sodium and 5.0 g of potassium per kg and drank distilled water (control). A second group of rats (n = 5) received a drinking solution containing 8.0 g NaCl per litre, and a third group of 5 rats received a diet containing virtually no sodium (less than 0.1 g per kg; Assia Maabaroth, Israel) and drank distilled water.

At the end of a dietary period of 14 days, the 24-hour urine was collected. The animals were then anaesthetized with Nembutal (60 mg/kg body weight, intraperitoneally). After laparotomy, the kidneys were perfused with cold heparinized isotonic NaCl solution and rapidly removed. The cortex was dissected and minced to a paste-like consistency. The homogenate was pushed through a 106 micron sieve which retained the tubules, then sieved through a 75 micron sieve which retained the glomeruli. Isolated cortical tubules, recovered from the sieve, were resuspended in Tris-HCl buffer and centrifuged twice at 120 *g* for 90 s. In light microscopy, these suspensions contain 70–80% of intact proximal and distal tubular fragments. After the last centrifugation, the supernatants were discarded and the pellets resuspended in Tris-HCl buffer, supplemented with $CaCl_2$ (5 mmol/l). The red outer medulla and the cone-shaped white papilla protruding in the pelvis were cut into 8–10 slices and suspended in the same buffer.

For the measurement of prostaglandin production, suspensions from one kidney were incubated in duplicate in a shaking bath at 37 °C for 30 min. The incubation was stopped by immersion of the tubes in ice-cold water. The tubes were then centrifuged for 10 minutes at 3000 *g* at 4 °C and supernatants stored at −70 °C until they were analysed for prostaglandin E_2 and prostaglandin $F_{2\alpha}$. Cortex, medulla and papilla from the contralateral kidney were homogenized, ultracentrifuged at 100 000 *g* and prostaglandin E_2 9-ketoreductase activity measured as previously described (9).

Sodium was measured by flame photometry. prostaglandin E_2 and prostaglandin $F_{2\alpha}$ were measured in the unextracted supernatants (10) by radioimmunoassay as previously described (11). The antisera, obtained from the Institut Pasteur, Paris, have negligible cross-reactivity with other prostanoids. Protein was measured by the method of *Lowry* et al. (12). Results are expressed as mean ± the standard error of the mean, and differences between the groups were studied by analysis of variance and a *t*-test for unpaired results.

Results

The influence of changes in sodium intake on its excretion is shown in table 1.

Tab. 1. Effect of changes in sodium intake.

	Low sodium	Control	High sodium
Urine volume (ml/day)	17 ± 2	22 ± 1	34 ± 4
Sodium output (mmol/day)	0.05 ± 0.01	1.9 ± 0.1	5.8 ± 0.6*

* $p < 0.01$ as compared with control

The synthesis of prostaglandin E_2 in isolated cortical tubules was not significantly influenced by changes in sodium balance. Prostaglandin $F_{2\alpha}$ increased with sodium depletion and the prostaglandin E_2/prostaglandin $F_{2\alpha}$ ratio decreased significantly. Prostaglandin E_2 9-ketoreductase activity was similar in the three groups. In contrast, the production of prostaglandin E_2 by medullary slices was decreased in both loaded and depleted rats. The synthesis of prostaglandin $F_{2\alpha}$ was unchanged. In both groups the prostaglandin E_2/prostaglandin $F_{2\alpha}$ ratio and prostaglandin E_2 9-ketoreductase activity were significantly decreased. In papillary slices, prostaglandin E_2 production increased with sodium depletion. A significant but smaller increase in prostaglandin E_2 production was observed in papillary slices from sodium-loaded rats. Prostaglandin $F_{2\alpha}$, on the other hand, was decreased with loading but did not change significantly with depletion. The prostaglandin E_2/prostaglandin $F_{2\alpha}$ ratio increased significantly in both groups as compared with controls, whereas prostaglandin E_2 9-ketoreductase was increased only with sodium depletion (tab. 2).

Discussion

Reduction of prostaglandin E_2 to prostaglandin $F_{2\alpha}$, which is catalysed by prostaglandin E_2 9-ketoreductase, is a catabolic pathway of potential physiological importance, since the relative potencies of the two substances in the kidney are quite different (5, 13). Early results generated considerable interest in the possible physiological role of this enzyme, and demonstrated the influence of dietary sodium intake on its activity (6, 8). It was therefore suggested that the levels of the more active prostaglandin E_2 could be altered by changes in sodium balance. Changes in prostaglandin E_2 levels could influence renin secretion (6) and sodium transport in different portions of the nephron (2, 4, 13). The concept of the prostaglandin E_2/prostaglandin $F_{2\alpha}$ ratio as a key factor in determining the net effect of the prostanoids on target organs was introduced (4, 6).

Others, however, have questioned the real importance of prostaglandin E_2 9-ketoreductase, on the basis of two major considerations:

Tab. 2. Production of prostaglandin E_2 and prostaglandin $F_{2\alpha}$ (pg/30 min · mg dry weight) and prostaglandin E_2 9-ketoreductase activity (prostaglandin $F_{2\alpha}$, ng/min · mg protein at 37 °C) in rats with different sodium intake.

	Low sodium	Control	High sodium
Cortex			
Prostaglandin E_2	179 ± 28	250 ± 35	165 ± 19
Prostaglandin $F_{2\alpha}$	115 ± 16**	61 ± 9	73 ± 9
Prostaglandin E_2/prostaglandin $F_{2\alpha}$ ratio	1.5 ± 0.4*	4.3 ± 1.3	2.4 ± 0.6**
Prostaglandin E_2 9-ketoreductase	1.9 ± 1.6	1.4 ± 0.6	1.6 ± 0.4
Medulla			
Prostaglandin E_2	23 ± 5*	115 ± 14	29 ± 4*
Prostaglandin $F_{2\alpha}$	29 ± 9	29 ± 3	30 ± 3
Prostaglandin E_2/prostaglandin $F_{2\alpha}$ ratio	0.8 ± 0.2*	4.1 ± 0.9	0.9 ± 0.2*
Prostaglandin E_2 9-ketoreductase	3.6 ± 0.9*	6.3 ± 1.4	3.2 ± 0.9*
Papilla			
Prostaglandin E_2	4415 ± 586*	1371 ± 99	2666 ± 416*
Prostaglandin $F_{2\alpha}$	1973 ± 464	2470 ± 141	1181 ± 104*
Prostaglandin E_2/prostaglandin $F_{2\alpha}$ ratio	2.3 ± 0.3**	0.5 ± 0.1	2.2 ± 0.8**
Prostaglandin E_2 9-ketoreductase	9.9 ± 3.9**	5.1 ± 1.2	3.5 ± 2.6

Values are expressed as mean ± standard error of the mean.
Compared with values of control groups: * = $p < 0.01$ and ** = $p < 0.05$

1) prostaglandin $F_{2\alpha}$ in the kidney might be derived from prostaglandin H_2 rather than from prostaglandin E_2 (7), and

2) the K_m of prostaglandin E_2 9-ketoreductase for prostaglandin E_2 is very high, well above the substrate concentrations usually found in vivo (7).

With regard to this last point, it should be noted that we have recently demonstrated that the K_m value of prostaglandin E_2 9-ketoreductase is much lower in rat papillary homogenates than that found in other kidney compartments, suggesting that the enzyme from this compartment of the nephron could play a regulatory role (9).

In the present investigation, we measured the synthesis of prostaglandins by different structures of the nephron in vitro, after manipulation of the sodium balance in vivo. We assume that the differences between the groups reflect mainly chronic modifications of prostaglandin production induced by the experimental protocol. As the major prostaglandin-producing compartments of the nephron have been well characterized, we may also assume that prostanoids derive mainly from cortical collection tubules in the cortical suspensions and from collecting tubules and interstitial cells in medullary and papillary slices (5).

In cortical and medullary preparations, the prostaglandin E_2/prostaglandin $F_{2\alpha}$ ratio was decreased during sodium depletion. However, this was due to both an increased prostaglandin $F_{2\alpha}$ synthesis in the cortex and a decreased prostaglandin E_2 synthesis in the medulla. Considering that prostaglandin E_2 inhibits sodium reabsorption in cortical and medullary collecting ducts (2, 13), these changes could well represent a homeostatic response. During sodium loading, prostaglandin production did not change in cortical preparations, whereas the changes in the medullary preparations were similar to those induced by sodium depletion. The physiological significance of these results is not clear at present. On the other hand, prostaglandin E_2 9-ketoreductase activity was not influenced by changes of sodium balance in cortical preparations. In the medulla, a similar decrease in prostaglandin E_2 9-ketoreductase activity was observed with both depletion and loading of sodium. Our findings are at variance with the results of previous studies in the rabbit, which have shown that both cortical and medullary prostaglandin E_2 9-ketoreductase levels increased with sodium loading (9). Our new data suggest that, at least in the rat, prostaglandin E_2 9-ketoreductase is not a major factor in the regulation of prostaglandin levels in these compartments of the nephron.

In the papilla, prostaglandin E_2 production was greatly increased during sodium depletion. Different factors, such as angiotensin II, bradykinin, antidiuretic hormone, as well as changes in osmolality, could all stimulate arachidonic acid release from phospholipids and, consequently, prostaglandin E_2 synthesis (5). In contrast, all of the above stimuli are depressed during sodium loading. A possible explanation for the stimulation of prostaglandin E_2 synthesis

with sodium loading is the increased tissue oxygen content, which is induced by increased papillary blood flow (14). Prostaglandin E_2 9-ketoreductase activity in papillary homogenates increased with sodium depletion and decreased with sodium loading. Again, this trend is in the opposite direction from the results on prostaglandin E_2 9-ketoreductase levels which have been reported by *Weber* et al. in rabbits (6). In addition, the physiological implication is unclear, as one would have expected an inverse correlation with the changes in the prostaglandin E_2/prostaglandin $F_{2\alpha}$ ratio.

In conclusion, the results of the present study in the rat are at variance with the findings obtained in the rabbit. Whereas prostaglandin E_2 seems to be important in the modifications of sodium transport induced by changes in sodium balance, due to its natriuretic effect, regulation of its levels by prostaglandin E_2 9-ketoreductase-mediated conversion to prostaglandin $F_{2\alpha}$ does not seem to play a major role in the process. It is thus possible that, in the rat, other renal enzymes which cause catabolism (and inactivation) of prostaglandin E_2, such as 15-hydroxyprostaglandin-dehydrogenase (NAD^+ or $NADP^+$)[1]) (15), or prostaglandin H_2 E isomerase[1]) (16), which is responsible for the conversion from prostaglandin H_2 to prostaglandin E_2, might be subject to regulation by changes of sodium balance.

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